Diagnosing multibacillary leprosy: A comparative evaluation of diagnostic accuracy of slit-skin smear, bacterial index of granuloma and WHO operational classification

Premanshu Bhushan, Kabir Sardana, R. V. Koranne, Monisha Choudhary[1], Prateek Manjul[2]

Departments of Dermatology, Leprosy and Sexually Transmitted Diseases, Lady Hardinge Medical College and Associated Hospitals, New Delhi, [1]Department of Pathology, Lady Hardinge Medical College and Associated Hospitals, New Delhi, [2]Resident, Skin Institute and School of Dermatology, New Delhi, India

Address for Correspondence: Dr. Premanshu Bhushan, C-12/436, Yamuna Vihar, Delhi-53, India. E-mail: drpremanshu@gmail.com

ABSTRACT

Background: In view of the relatively poor performance of skin smears WHO adopted a purely clinical operational classification, however the poor specificity of operational classification leads to overdiagnosis and unwarranted overtreatment while the poor sensitivity leads to underdiagnosis of multibacillary (MB) cases with inadequate treatment. Bacilli are more frequently and abundantly demonstrated in tissue sections. **Aims and Methods:** We compared WHO classification, slit-skin smears (SSS) and demonstration of bacilli in biopsies (bacterial index of granuloma or BIG) with regards to their efficacy in correctly identifying multibacillary cases. The tests were done on 141 patients and were evaluated for their ability to diagnose true MB leprosy using detailed statistical analysis. **Results:** A total of 76 patients were truly MB with either positive smears, BIG positivity or with a typical histology of BB, BL or LL. Amongst these 76 true-MB patients, WHO operational classification correctly identified multibacillary status in 56 (73.68%), and SSS in 43 (56.58%), while BIG correctly identified 65 (85.53%) true-MB cases. **Conclusion:** BIG was most sensitive and effective of the three methods especially in paucilesional patients. We suggest adding estimation of bacterial index of granuloma in the diagnostic workup of paucilesional patients.

Key Words: Bacterial index, Bacterial index of granuloma, Leprosy diagnosis

INTRODUCTION

The sixth WHO expert committee report in 1988 recommended all smear positive leprosy cases be treated as MB patient[1] which was later changed to a purely clinical classification with patients having >5 skin lesions considered MB and ≤5 as PB.[2] However, such purely clinical classification leads to a small but significant number of MB cases being treated with PB regimen.[3] The specificity of slit-smears is almost 100% as it directly demonstrates the presence of acid-fast bacilli (AFB) but the sensitivity is low and varies from 10-50%.[3] Histological examination has many advantages and the yield of AFB in tissue sections are reported to be better.[4-6] The study was designed to compare the efficacy of bacterial index (BI) in SSS and BIG in biopsies for detecting the truly MB cases and the relative performance of WHO operational classification, SSS and BIG in tissue sections in this respect.

METHODS

We studied 150 consecutive and untreated cases of leprosy at the Department of Dermatology, Lady Hardinge Medical College and associated hospitals, New Delhi. Patients who had received any specific therapy for leprosy in the past, those who had pure neuritic leprosy or those who did not give consent were excluded from the study.

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Patients were clinically classified based on number, type and characteristics (including grade of sensory loss, borders, dryness, scales, hair-loss etc.) of skin lesions and nerve involvement; into indeterminate (I), tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) and lepromatous (LL) leprosy. Patients were also classified as PB or MB based on the number of skin lesions. Three slit skin smears, two from the representative lesions and one from an earlobe were obtained and stained by Ziehl Neelsen method. At least 100 oil-immersion fields of the smears were examined by at least two investigators for the presence of AFB and BI was calculated. Skin biopsies from most representative lesions were stained by both hematoxylin and eosin as well as modified Fite method[7] for AFB.[7] A minimum of three complete tissue sections were examined by at least two of the investigators for histopathological changes and for calculation of the BIG in them.[8,9] For both BI and BIG calculation two investigators examined the slides simultaneously and a consensus value was taken. We did not analyze for inter-observer variations. Patients who demonstrated AFB in SSS or biopsy as well as those with a typical histology of BB, BL or LL were considered *true*-MB patients.[5] The data thus obtained was pooled and analyzed with Statistical Package for Social Sciences (SPSS™ for Windows™ V 9.0.0, SPSS, Inc.) and Smith's Statistical Package™ V 2.75.

RESULTS

Study group

Of the 150 patients originally included for study, in 9 patients skin biopsy was impossible to cut or stain properly and these were not analyzed in study.

Clinical features

The mean age was 28.709 (± 13.851 years). The study group consisted of 102 (72.34%) males and 39 (27.66%) females. The male: female ratio was 2.61: 1. Forty-two (29.79%) patients had a single skin lesion (SSL), 25 (17.73%) patients had 2-5 lesions, and 74 (52.48%) had >5 lesions. Thus 67 (47.5%) patients were PB as per WHO classification while 74 (52.2%) were MB. The maximum number of patients clinically belonged to BT, which constituted 83 (58.86%) patients. There were 23 (16.31%) patients of BL, 12 (8.51%) each of BB and LL, 3 of TT and 8 of indeterminate types of leprosy.

Skin slit smears

The slit skin smears (SSS) were positive in 43(30.50%) cases. Amongst them, 29 were from BL and LL patients, while 9 were from BB and 5 from BT patients. All the smears were negative in indeterminate and tuberculoid patients. The mean BI was 0.943 [SD: 1.638].

Histological examination

The overall concordance in clinical and histological diagnosis was observed in 105 (74.47%) cases. The concordance was maximum in LL (12) and TT (3) cases with 100% agreement, and was 69 (83.13%) in BT, 6 (50%) in BB, and 15 (65.22%) in BL cases.

BIG in tissue sections

Overall 65 (46.09%) patients showed BIG positivity. The mean BIG was 1.645 [SD: 2.098]. No AFB were seen in indeterminate and TT patients. The AFB were detected in tissue sections of 29 (34.94%) of BT, 8 (66.67%) of BB, 18 (78.26%) BL and 10 (83.33%) LL patients. The BI and BIG in different types of leprosy is presented in Table 1.

Table 1: Comparative performance of slit skin smears for bacterial index and skin biopsy for bacterial index of granuloma

Clinical type of leprosy	Diagnosis											
	I		TT		BT		BB		BL		LL	
Number of patients	8		3		83		12		23		12	
Value	BI	BIG	BI	BIG	BI	BIG	BI	BIG	BI	BIG	BI	BIG
0+	8	8	3	3	78	54	3	4	5	5	1	2
1+					5	8	3	1				
2+						8	3	2	3	1		1
3+						4	3	3	9	1	3	1
4+						3		2	3	4		
5+						6			3	12	7	2
6+											1	6
Mean	0	0	0	0	0.06	0.94	1.50	1.83	2.61	3.52	4.17	4.25
Standard deviation	0	0	0	0	0.24	1.56	1.17	1.59	1.64	2.04	1.64	2.38
P value of difference in BI and BIG	NA		NA		<0.0001		0.5684		0.10		0.9245	

BIG: Bacterial index of granuloma, BI: Bacterial index, TT: Tuberculoid, BT: Borderline tuberculoid, BB: Borderline borderline, BL: Borderline lepromatous, LL: Lepromatous

Comparison of SSS and BIG [Table 1 and Figure 1]

As compared to SSS (43 i.e. 56.58%) the BIG identified significantly more number of MB cases (65 i.e.85.53%) [Chi-square test, $p<0.001$]. There were 32 (22.70%) patients, who had AFB in biopsies but not in the SSS, including 27 BT, 4 BL and 1 LL cases. All TT and indeterminate patients were negative on both SSS and biopsy for AFB. Five (6.02%) of 83 BT patients were BI positive while 29 (34.94%) of them revealed AFB in biopsy sections with 27 of these 29 patients having negative SSS. Surprisingly, 5 patients (3 BT, 1BB and 1 LL) had AFB in smears but not on biopsies.

The difference between the values of BIG and BI varied from 0 to 6. The mean difference between the values of BI and BIG was higher than BI in all and it was least in LL [0.08 (± 0.71)] and maximum in BL [0.91 (± 0.55)]. The difference between the values (bacterial yield) of BI [mean: 0.943; SD: 1.638] and BIG [mean: 1.645; SD: 2.098] was statistically analyzed with *paired-t-test* and was found to be highly significant ($p<0.0001$). Furthermore, the difference was highly significant in BT patients with $p < 0.0001$). It was also observed that 33 (76.74%) out of 43 SSS positive cases were also positive for BIG. In contrast, SSS was positive only in 33 (50.77%) out of 65 patients who were BIG positive and the difference was again significant (Chi-square test, $p< 0.001$).

The SSS were positive in 2(2.98%) of the 67 WHO-PB patients [1 SSL and one with 2-5 lesions] as compared to 18 (26.87%) BIG positive patients [5 SSL and 13 with 2-5 lesions] and the difference was significant (*z-test;* $p<0.05$). Again, BIG was positive in 47 of 74 (63.51%) WHO-MB patients as compared to 41 (55.40%) SSS positive cases however, the difference was statistically not significant.

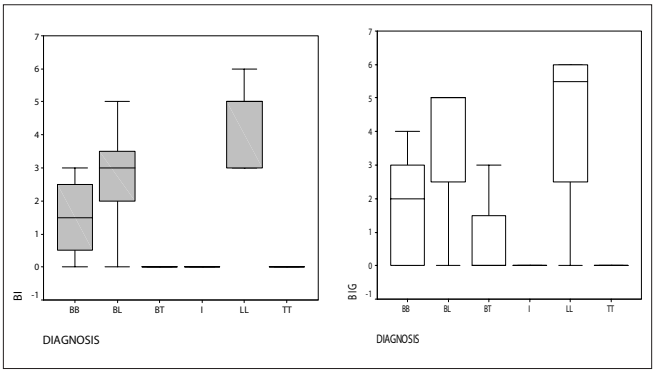

Figure 1: Comparative performance of BI and BIG in different types of leprosy: The left chart with gray boxes is for BI while the right chart with white boxes depicts BIG in different types of leprosy. (The box plot showing the minimum data value, the lower quartile, the median, the upper quartile, and the maximum data value on a number line. A box is drawn from the lower quartile to the upper quartile. The median is marked inside the box with a line.)

Amongst 26 highly bacillated patients, i.e., with a BIG of 5-6+, SSS were positive for AFB in 17 (65.38%) patients. On the other hand, for BIG values of 1-4+ the SSS were positive in 16 out of 39 (41.02%) patients. Since SSS was going to be more positive in highly bacillated patients only, *one tailed Fisher's exact test*[10] was conducted and the difference was statistically significant ($p<0.05$). Therefore, at low tissue bacillary density BI was far less effective in demonstrating AFB.

Performance of WHO classification as compared to SSS and BIG

SSS correctly identified MB patients in 43 of 76 *true*-MB cases with a sensitivity of 56.58%, while skin biopsy showed AFB in 65 of 76 *true*-MB cases with sensitivity of 85.53%. The specificity and the positive predictive value (PPV) for both BI and BIG would naturally be 100% as they directly demonstrate AFB; however the negative predictive value (NPV) of SSS was 66.33% as compared to 85.53% of BIG.

WHO classification alone, correctly classified 56 of 76 *true*-MB patients with a sensitivity of 73.68% and specificity of 72.31%. Thus 26.32% patients were misclassified as PB. The PPV of WHO system was 75.68% and the NPV was 70.15%. The *McNemar's tests*[10] for the differences between sensitivities of SSS, BIG and WHO system were significant ($P<0.001$). Interestingly the WHO classification classified 18 (27.69%) of 65 *true*-PB patients as MB thus leading to overdiagnosis. WHO classification in conjunction with SSS would still have misclassified 18 of 76 true MB patients as PB i.e., skin biopsies and BIG identified 23.68% of misclassified PB cases as *true*-MB cases.

Further, the concordance between BIG, SSS and WHO system to correctly identify *true*-MB patients was evaluated by the *Cohen's Kappa coefficient*[10] and concordance was deemed to be mediocre when it was <0.40 and good when ≥0.60. The *Cohen's Kappa coefficient was* 0.845 for BIG, 0.546 for SSS and 0.459 for WHO classification. The mean BI in *true*-MB patients was 1.75(± 1.89) while the mean BIG in *true*-MB patients was 3.05(± 1.97). The difference between the mean BI and BIG was analyzed with *two sided t test* and the result was statistically significant ($P<0.001$).

DISCUSSION

Demonstrating AFB is still considered important for diagnosis, classification and management of leprosy.[11,12]

However, the sensitivity of SSS is poor (10-50%)[3] and it has been described as the weakest link.[13] To overcome the shortcomings of smears, WHO proposed a purely clinical classification.[2] Despite routine use, this classification has reported sensitivity from 85-93% with specificity of 42-88%.[14-19] Further addition of more clinical criteria does not increase the sensitivity and specificity.[18,19] While AFB are better demonstrated in biopsies,[4-6] it is technically demanding, invasive and has no definite role in management of leprosy. The reducing prevalence of leprosy entails an incremental involvement of higher centers.[2,6] We evaluated whether additional information from BIG would increase the diagnostic accuracy in identifying MB patients.

In our study significantly more patients were identified as MB with BIG(46.09%) as compared to SSS(30.50%). This BIG positivity in SSS negative patients is explained by the presence of AFB in deep reticular dermis where they remain inaccessible to SSS.[20] Similar findings of better performance of biopsy were reported in various studies.[6,21-24] We have demonstrated that this high positive BIG is also significantly seen in paucilesional, clinically BT and WHO-PB patients. Ponnighaus *et al,* reported finding 2 of 61 SSL patients with AFB-negative smears yet positive biopsies.[25] Similar observation was made by Srinivaas *et al,*[6] Our analysis reconfirmed earlier findings that in highly bacillated patients, SSS is quite sensitive but not in patients with low tissue-density of AFB.[6,25] Therefore, SSS has significant underdiagnosis of *true*-MB patients.

However, in 5 (11.63%) of our SSS positive patients BIG was negative. Groenen *et al,* also reported that 15% of patients were BI positive but BIG negative.[5] The explanation for this contradictory observation is that bacilli in biopsy may be missed because the biopsy is taken at the wrong spot or because the bacilli are concentrated in one specific area but the biopsy slices do not include the area.[26,27]

SSS in our study had a low sensitivity and NPV as compared to BIG. Such low sensitivity of SSS has been deliberated before,[3,18] while poor NPV indicates a large proportion of patients being misclassified as PB. Such poor performance of SSS at a tertiary center underscores the importance of WHO classification, as poorer results would be expected in peripheral centers.

Our analysis revealed the sensitivity to be higher with BIG as compared to WHO scheme or SSS. Further, the WHO system lead to overdiagnosis in 18 (27.69%) of 65 true-PB patients. Therefore, WHO system is significantly more sensitive than SSS but less than BIG and has the drawback of significant underdiagnosis as well as overdiagnosis. Many studies have demonstrated the sensitivity of WHO operational classifications ranging from 85-92%[14-19] but many of these high values were observed using slit-smear as the "gold-standard".[14-16,18] Ideally a skin or nerve biopsy should have been included to make diagnosis of *true*-MB leprosy.[5,19] WHO system increases the sensitivity significantly as compared to SSS, but at a cost of a poor specificity as reported to be 41.3% by Groenen *et al*.[5] Even a combination of SSS with WHO operational classification added little to the sensitivity and still resulted in 23.68% underdiagnosis of *true*-MB cases as compared to BIG. The concordance analysis also demonstrated that BIG most closely approximated the *true*-MB status compared to SSS and WHO classification.

Thus, WHO system is better than over-reliance on SSS, but still leads to significant underdiagnosis, undertreatment and consequent risks of resistance, relapses and progressive horizontal transmission. This is especially true of paucilesional patients and clinically BT patients. BIG is the most superior method for detecting AFB in leprosy patients and most closely resembles the true bacteriological status.

We propose that in view of good sensitivity of WHO classification for patients with > 5 lesions, it may be worthwhile to consider doing histological analysis and BIG estimation in paucilesional patients, if the facilities are available. Importantly, one does not need to count the bacilli for managing patients and finding even one AFB in tissue section will be enough to consider a patient as MB, thus making the process easier. Conversely, the need for BI/BIG estimation can be done away with, if a uniform-MDT with three drugs were accepted in control programmes.[28]

REFERENCES

1. World Health Organization Expert Committee on Leprosy. 6th report. Geneva: World Health Organization; 1988. p. 768.
2. World Health Organization Expert Committee on Leprosy. 7th report. Geneva: World Health Organization; 1998. p. 874.
3. Diagnosis and Classification of Leprosy. In: Report of the International Leprosy Association Technical Forum 2002. Lepr Rev 2002;73:S17-26.
4. Croft RP, Smith WC, Nicholls P, Richardus JH. Sensitivity and specificity of methods of classification of leprosy without use of skin smear examination. Int J Lepr 1998;66:445-50.
5. Groenen G, Saha NG, Rashid MA, Hamid MA, Pattyn SR. Classification of Leprosy Cases under Field Conditions in Bangladesh: I, Usefulness of Skin-Smear Examination. Lepr Rev 1995;66:26-33.

6. Srinivas D, Rao PN, Lakshmi TS, Suneetha S. Bacterial index of granuloma and its relevance compared to BI of skin smears. Lepr Rev 2002;73:79-80.
7. Raphel SS. Routine staining of sections. In: Lynch's medical laboratory technology, 3rd ed. W.B. Saunders Company; 1976. p. 916-33.
8. Ridley DS, Hilson GR. A logarithmic index of bacilli in biopsies: I, Method. Int J Lepr Other Mycobact Dis 1967;35:84-6.
9. Ridley DS. A logarithmic index of bacilli in biopsies: 2, Evaluation. Int J Lepr Other Mycobact Dis 1967;35:187-93.
10. Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley and Sons; 1981.
11. Waters MF. To smear or not to smear? Lepr Rev 2002;73:211-4.
12. Georgiev GD, Mcdougall AC. A reappraisal of clinical and histological criteria in the implementation of MDT for leprosy control programmes and proposal for their better use. Lepr Rev 1990;64:64-72.
13. Directorate of Health Services, India. Leprosy Division. National Leprosy Eradication Programme. New Delhi: Report of Independent Evaluation; 1986.
14. Becx-Bleumink M. Allocation of patients to paucibacillary or multibacillary drug regimens for the treatment of leprosy: A comparision of methods based mainly on skin smears as opposed to clinical methods-alternative clinical methods for classification of patients. Int J Lepr Other Mycobact Dis 1991;59:292-303.
15. Croft RP, Smith WC, Nicholls P, Richardus JH. Sensitivity and specificity of methods of classification of leprosy without use of skin smear examination. Int J Lepr Other Mycobact Dis 1998;66:445-50.
16. Buhrer-Sekula S, Sarno EN, Oskam L, Koop S, Wickers I, Nery JA, *et al*. Use of ML dipstick as a tool to classify leprosy patients. Int J Lepr Other Mycobact Dis 2000;68:456-63.
17. Dasananjali K, Schreuder PA, Pirayavaraporn C. A study on the effectiveness and the safety of the WHO/MDT regimen in the Northeast of Thailand: A prospective study, 1984-1996. Int J Lepr Other Mycobact Dis1997;65:28-36.
18. Groenen G, Saha NG, Rashid MA, Hamid MA, Pattyn SR. Classification of Leprosy Cases under Field Conditions in Bangladesh, II. Lepr Rev 1995;66:134-43.
19. Norman G, Joseph G, Richard J. Validity of the WHO operational classification and value of other clinical signs in the classification of leprosy. Int J Lepr Other Mycobact Dis 2004;72:278-83.
20. Suneetha S, Arunthathi S, Chandi S, Kurian N, Chacko CJ. Histological studies in primary neuritic leprosy changes in apparently normal skin. Lepr Rev 1998;69:351-7.
21. Ridley DS. The Bacteriological Interpretation of Skin Smears and Biopsies in Leprosy. Trans R Soc Trop Med Hyg 1955;49:449-52.
22. Nayak SV, Shivarudrappa AS, Nagarajappa AH, Sacchidanand S, Ahmed SM. Role of modified lipid AFB method in histopathological sections of Hansen's disease. Indian J Dermatol Venereol Leprol 2003;69:173-4.
23. Van Brakel WH, De Soldenhoff R, Mcdougall AC. The allocation of leprosy patients into paucibacillary and multibacillary group for multidrug therapy, taking in account the no of body areas affected by skin, or skin and nerve lesions. Lepr Rev 1992;63:231-45.
24. Fleury RN, Aranda CM. Detection of AFB in tuberculoid biopsies. Int J Lepr Other Mycobact Dis 1995;63:103.
25. Ponnighaus JM, Lienhardt C, Lucas S, Fine PE, Sterne JA. Comparison of Bacillary Indexes in Slit-Skin Smears, Skin, and Nerve Biopsies: A Study from Malawi. Int J Lepr Other Mycobact Dis 1997;65:211-6.
26. Lucas SB, Ridley DS. The use of histopathology in leprosy diagnosis and research. Lepr Rev 1989;60:257-62.
27. Vettom L, Pritze S. Reliability of skin smear results: Experiences with quality control of skin smears in different routine services in leprosy control progammes. Lepr Rev 1989;60:187-96.
28. Sardana K, Koranne RV, Mahajan S, Bhushan P. Correlation of bacterial index (BI) and bacterial index of granuloma (BIG) in leprosy-is there a therapeutic relevance? Indian J Lepr, 2004;76(4):363-9.